

Appl. No. 09/643,755  
Amdt. Dated September 28, 2005  
Reply to Office action of June 28, 2005

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claim 1 (Currently amended): A method for the production of chymosin in a plant seed comprising an oil fraction comprising:

- a) introducing into a plant cell a chimeric nucleic acid sequence molecule comprising in the 5' to 3' direction of transcription:
  - 1) a seed-specific promoter capable of regulating transcription in said plant cell operatively linked to;
  - 2) a second nucleic acid sequence encoding a chymosin polypeptide operatively linked to;
  - 3) a third nucleic acid sequence capable of terminating transcription in said plant cell;
- b) growing said plant cell into a mature plant capable of setting seed wherein said seed contains chymosin;
- c) obtaining seed from the mature plant wherein the seed contains at least 0.5% (w/w) chymosin; and
- d) isolating said chymosin from said seed using a method comprising:
  - (i) crushing the plant seed to obtain crushed plant seed;
  - (ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material;
  - (iii) contacting the aqueous fraction with a protein binding resin; and
  - (iv) recovering chymosin from the protein binding resin such that said chymosin is purified to homogeneity.

Claim 2 (cancelled).

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**Claim 3 (previously presented):** The method according to claim 1 wherein said seed-specific promoter is a phaseolin promoter.

**Claim 4 (cancelled).**

**Claim 5 (original):** The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide comprises a nucleic acid sequence encoding a chymosin pro-peptide, a nucleic acid sequence encoding a chymosin pre-peptide or a nucleic acid sequence encoding chymosin pre-pro-peptide.

**Claim 6 (original):** The method according to claim 5 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

**Claim 7 (original):** The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

**Claim 8 (previously presented):** The method according to claim 7 wherein the plant signal sequence is a tobacco PR-S signal sequence.

**Claim 9 (original):** The method according to claim 8 wherein the nucleic acid sequence encoding chymosin linked to a PR-S signal sequence comprises a nucleic acid sequence as in SEQ.ID.NO.:1.

**Claim 10 (original):** The method according to claim 1 wherein said third nucleic acid sequence is a phaseolin terminator.

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**Claim 11 (previously presented):** The method according to claim 1 wherein the chymosin is a mammalian chymosin obtained from a bovine, sheep or goat source.

**Claim 12 (original):** The method according to claim 6 wherein codon usage for said nucleic acid sequence encoding chymosin, chymosin pro-peptide, chymosin pre-peptide and chymosin pre-pro-peptide has been optimized for use in plants.

**Claim 13 (original):** The method according to claim 1 wherein said plant is selected from the group of plants consisting of soybean (*Glycine max*), rapeseed (*Brassica napus*, *Brassica campestris*), sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), alfalfa (*Medicago sativa*), wheat (*Triticum sp.*), barley (*Hordeum vulgare*), oats (*Avena sativa* L.), sorghum (*Sorghum bicolor*), Arabidopsis thaliana, potato (*Solanum sp.*), flax/linseed (*Linum usitatissimum*), safflower (*Carthamus tinctorius*), oil palm (*Eleais guineensis*), groundnut (*Arachis hypogaea*), Brazil nut (*Bertholletia excelsa*) coconut (*Cocus nucifera*), castor (*Ricinus communis*), coriander (*Coriandrum sativum*), squash (*Cucurbita maxima*), jojoba (*Simmondsia chinensis*) and rice (*Oryza sativa*).

**Claim 14 (currently amended):** The method according to claim 1 wherein at least 1% (w/w) of ~~said~~-total seed protein of said seed is chymosin.

**Claim 15 (currently amended):** The method according to claim 1 wherein at least 2% (w/w) of ~~said~~-total seed protein of said seed is chymosin.

**Claim 16 (currently amended):** The method according to claim 1 wherein at least 4% (w/w) of ~~said~~-total seed protein of said seed is chymosin.

**Claim 17 (Currently amended):** A method for the production of plant seeds comprising an oil fraction containing at least 0.5% (w/w) chymosin in the total seed protein comprising:

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(a) introducing into each of at least two plant cells a chimeric nucleic acid sequence molecule comprising in the 5' to 3' direction of transcription:

- 1) a seed-specific promoter capable of regulating transcription in said plant cell operatively linked to;
- 2) a second nucleic acid sequence encoding a chymosin polypeptide operatively linked to;
- 3) a third nucleic acid sequence capable of terminating transcription in said plant cell;

(b) growing each plant cell into a mature plant capable of setting seed;

(c) obtaining seed from each mature plant;

(d) detecting the levels of chymosin in the seed of each plant obtained in step (c) or in the seed of a plant generated from the seed of a plant obtained in step (c);

(e) selecting plants that contain at least 0.5% (w/w) chymosin in the total seed protein; and

(f) isolating said chymosin from said seed using a method comprising:

- (i) crushing the plant seed to obtain crushed plant seed;
- (ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material;
- (iii) contacting the aqueous fraction with a protein binding resin; and
- (iv) recovering chymosin from the protein binding resin such that said chymosin is purified to homogeneity.

Claims 18-20 (cancelled).

Claim 21 (previously presented): A method according to claim 1 wherein said protein binding resin is a hydrophobic interaction resin.

Claim 22 (previously presented): A method according to claim 17 wherein said protein binding resin is a hydrophobic interaction resin.

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**Claim 23:** A method according to claim 22 further comprising using an ion exchange resin to further purify the chymosin.

**Claims 24-28 (cancelled).**